chapter 10

THE BIOSYNTHESIS OF PROTEINS

There can be little doubt that the specific information necessary for the biosynthesis of proteins is, in some way, woven into the structure of the deoxyribonucleic acids of the chromosome. Ample support for this conclusion is given by the numerous observations that have related Mendelian genes to individual protein molecules. As we have seen, the most direct evidence has come from instances in which the genetic results could be compared with the chemical and physical properties of isolated, homogeneous proteins, such as hemoglobin, tyrosinase, and β-lactoglobulin. Equally convincing are the results obtained by bacteriologists and virologists who have demonstrated that highly purified samples of DNA are capable of modifying both the genotype and phenotype of recipient cells, or of inducing the formation of the relatively complicated protein complex which characterizes the bacteriophage particle.

It is clear, however, that protein synthesis can take place outside the nucleus itself. In the reticulocyte, for example, hemoglobin synthesis proceeds at a rapid rate, and not until the cell has become a mature erythrocyte does such synthesis cease. Similarly, in the alga *Acetabularia mediterranea,* whose cell may be separated into nuclear

and anuclear halves, the latter fragment temporarily synthesizes protein at a rate even more rapid than that of the intact cell, although this synthetic activity soon disappears. If the biosynthesis of a specific, chemically definable protein like hemoglobin can continue in the absence of an intact nucleus, it becomes essential to focus our attention on the mechanism by which the requisite information might be transferred to, and perhaps temporarily stored in, the cytoplasm of cells.

The biosynthesis of protein is among the biological phenomena that are highly dependent on structural organization. Even when synthesis can proceed in the absence of a nucleus, the process is only temporarily maintained (although admittedly this degeneration might be due to deficiencies in any one of a number of metabolic factors only indirectly related to protein synthesis per se). Because of this dependence on structural integrity, the recent investigations of the nature of cellular substructure have contributed perhaps the most important advance toward an ultimate understanding of the nature of the biosynthetic mechanism. These studies, in spite of their emphasis on static morphology, are beginning to give us a picture of the cell as a highly organized system of interconnected metabolic units into which all the dramatic observations of the enzyme chemist and the geneticist must ultimately be fitted.

Two relatively new techniques—electron microscopy of ultrathin sections and differential sedimentation of cellular components in sucrose solutions—have been of particular importance in this process of the description of cellular architecture. The latter of these methods permits the isolation of more or less homogeneous samples of mitochondria, microsomes, nuclei, and other cell inclusions, allowing the study of the relative abilities of these particulate fractions to incorporate labeled precursors into nucleic acids and proteins. We shall discuss these observations later, but first let us look at some of the results, obtained through electron microscopy, which indicate how these functional components are arranged within the intact cell.

The electron micrograph in Figure 90, taken by Dr. George Palade of the Rockefeller Institute, is of guinea pig pancreas. Careful examination and measurement of many such photographs have established the presence in the cytoplasm of concentrically arranged membranes, having a thickness of about 40 A. These membranes, variously called the "endoplasmic reticulum," the "ergastoplasm," or simply the "intracellular cytoplasmic membrane," are studded throughout with small electron-dense granules. These are the gran-

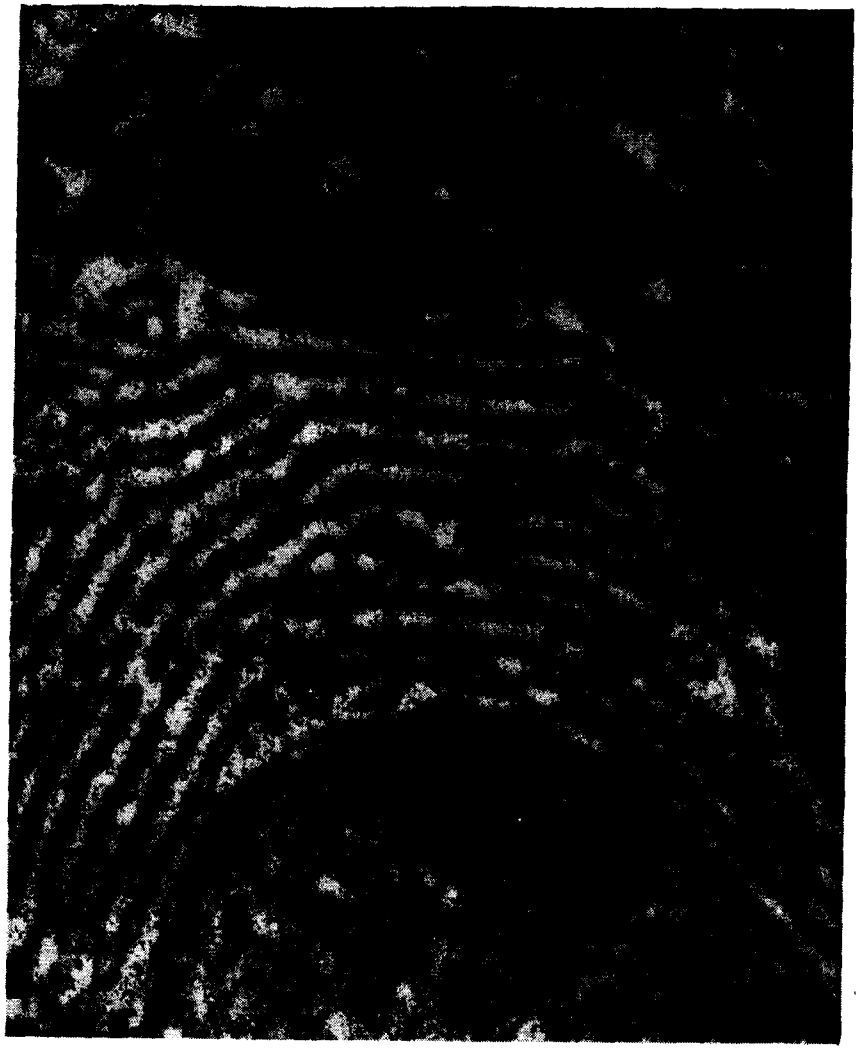

Figure 90. Electron photomicrograph of a section through a cell from the pancreas of the guinea pig. Magnification, 56,000×. The layered structures at the center of the photograph are the "endoplasmic reticulum" and consist of lipoidal membranes studded with particles rich in RNA. The oval, cross-channeled structures at the top are mitochondria. A portion of the cell nucleus is visible at the bottom of the photograph. This photograph was obtained through the kindness of Dr. George Palade of the Rockefeller Institute for Medical Research. From "The Endoplasmic Reticulum," *J. Biophys. Biochem. Cytology*, **2**, (suppl.) 85 (1956).

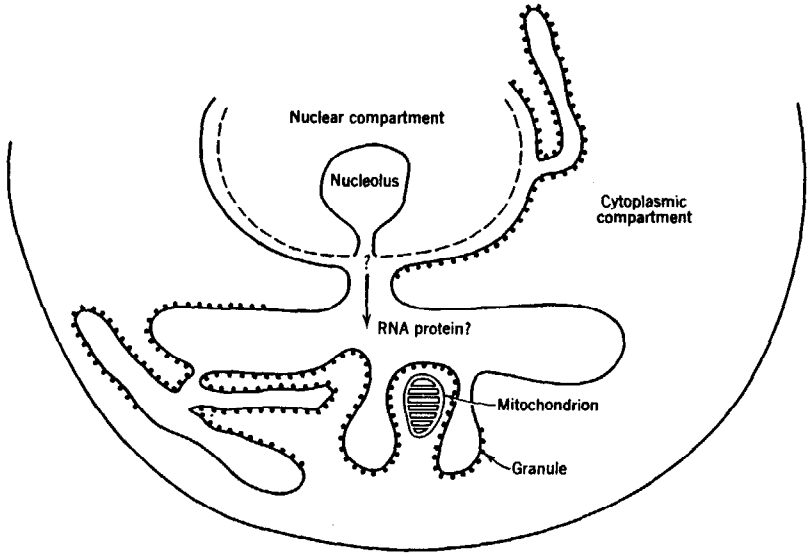

Figure 91. A schematic diagram of how some of the structural elements within a cell might be organized. The cytoplasm of the cell is visualized as being divided into two portions by the folded and invaginated endoplasmic reticulum, the "internal" portion being continuous with the outer surface of the nucleus and possibly directly connected with the nucleolus through a small channel. The "external" portion of the cytoplasm occupies a space between the endoplasmic reticulum and the plasma membrane of the cell and contains the mitochondria.

ules which, following homogenization of a tissue, may be isolated as a discrete fraction by differential centrifugation, attached to pieces of fragmented membrane. Sjöstrand and Hanzon[1] have reported that, in their experience (and this is generally confirmed by many investigators), the granule-studded membranes are always arranged so that they face the mitochondria, the cell membrane, or each other with their rough side, but that around the nucleus the smooth side of the membrane is presented. Such an arrangement would be compatible with the situation outlined schematically in Figure 91. Here the reticulum is visualized not as a number of independent membranes but as a large balloon-like structure surrounding the nucleus and crumpled upon itself. This would lead to the sort of orientation of granules suggested by Sjöstrand and Hanzon and would serve to divide the cell up into two major compartments, one containing the nucleus and the other the mitochondria, together with the cytoplasmic fluid in which they are bathed. Thus, the "crumpled balloon"

arrangement would furnish the cell with a large surface as a site for metabolic activity and would also serve as a natural divider of the "genetic" and "assembly line" portions of the cell.

It must be emphasized that the scheme presented in Figure 91 is only the distillation of several possibilities considered reasonable by expert cytologists. It is included here only to indicate to the reader the degree of sophistication that has been reached in the study of cellular substructure. Uniformity of interpretation by experts is more than can be expected in any rapidly advancing field, and it is gratifying that most of the differences of opinion among cytologists revolve around relatively minor points.

When a tissue is homogenized, the endoplasmic reticulum is disintegrated. From recent analyses it would appear that the so-called microsome fraction is composed mainly of granules to which pieces of reticulum still adhere. Thus, treatment of microsomal preparations with lipoprotein-disrupting agents such as deoxycholate yields particles containing most of the RNA of the original preparation but only a small proportion (about one-sixth) of the original protein. With ribonuclease, on the other hand, which digests and depolymerizes the RNA, only membranous material is observed upon electron microscopic examination. In some tissues, like the hen's oviduct, the ergastoplasm is not as friable, and, even after fairly vigorous homogenization, a relatively intact membrane particle complex may be isolated by centrifugation at relatively low speeds.

The origin of the ergastoplasm is not established. It has recently been shown that, in the hepatic cells of animals fed after a prolonged fasting period, regeneration of membranes begins near the periphery of the cell. These membranes are free of granules and only later assume the studded appearance which characterizes them in an actively secreting cell. It has been suggested that the reticulum might be the product of the continuing process of pinocytosis (water imbibition) and phagocytosis (particle imbibition) at the cell surface. Electron microscope studies have indicated that engulfed fluids and solids are surrounded with a sheath of the external plasma membrane of the cell, pinched off during the passage of nutrients across the cell surface. This membrane ultimately becomes continuous with the endoplasmic reticulum. The observations, if substantiated, would require some rather vigorous metabolism. For example, as Swerdlow, Dalton, and Birks[2] have recently pointed out, if the incorporation of the plasma membrane into actively imbibing cells like macrophages were continuous, the cells would soon consist of nothing else. Such cells would obviously require active processes both for the regenera-

tion of new plasma membrane and for the breakdown of reticulum as it accumulated and pressed in on the nucleus.

Cytological Aspects of Protein Synthesis

In 1940, T. Caspersson[3] suggested a general theory of protein biosynthesis which is, in essence, still in accordance with observation. In his theory the genetic information within the DNA of the nucleus was pictured as being transmitted to the nucleolus through the medium of basic nucleohistones, rich in arginine and lysine. The nucleolus then converted this information into ribonucleoprotein which, in turn, induced and directed the biosynthesis of proteins by enzyme systems in the cytoplasm.

One aspect of this theory can probably be safely discarded. The role postulated for the nucleohistones was one of precursor for the purines and pyrimidines of the ribonucleoprotein. We now know, from isotope studies, that the building blocks of RNA are simple substances such as glycine, formate, and carbon dioxide and are not basic amino acids. We cannot, however, rule out the possibility that nucleohistones might serve as agents of information transfer, acting, in a sense, as negative prints of the specifically arranged nucleotide sequences of DNA.

In this connection, Wilkins and his colleagues have suggested the interesting hypothesis that protamines and nucleohistones might be wrapped around the double helix of the DNA strand (shown in profile in Figure 92). This suggestion, admittedly based on very preliminary X-ray studies, is consistent with certain amino acid sequence data by Felix, Fischer, and Krekels.[4] About one-third of the residues in protamines are nonbasic, and these residues would have to occur at folds in the polypeptide chain to permit all basic side chains to associate with phosphate groups. The sequence analyses do in fact show that nonbasic residues occur in pairs and that folds such as those indicated in the figure would thus be possible.

What evidence can we marshal for the second step in Caspersson's theory, namely, that the nucleolus is concerned with the synthesis of cytoplasmic ribonucleoprotein? Here we find ourselves immersed in a mass of data, much of it conflicting. The fact, for example, that some enucleated cells can continue to form protein suggests that cytoplasm is autonomous in this respect. On the other hand, we may postulate that ribonucleoprotein, produced in the nucleus and containing genetic information, has a certain "life expectancy" in the

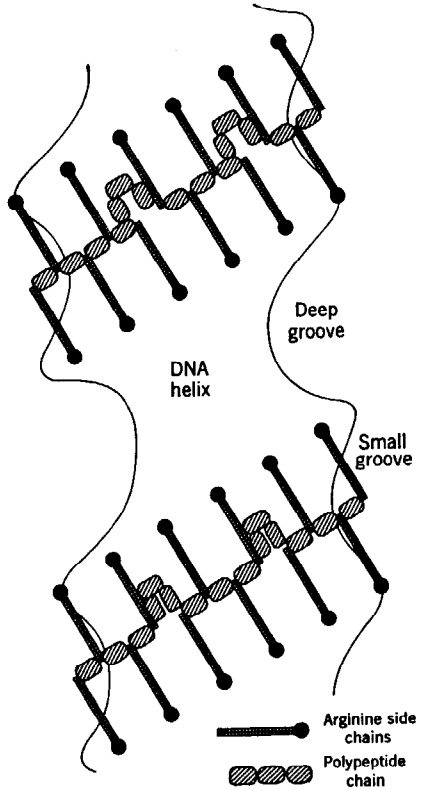

Figure 92. A diagram showing how protamine might be wrapped in a spiral fashion around the DNA double helix. The polypeptide chain is wound around the small groove of the helix. Phosphate groups of the DNA coincide with the basic ends of the arginine chains (black circles) of the protamine molecule. Nonbasic residues are shown in pairs at the folds in the polypeptide chain. From M. H. F. Wilkins, *Cold Spring Harbor Symposia Quant. Biol.*, **21**, 75 (1956).

cell which varies from species to species, and that protein synthesis may proceed as long as some of this material remains functional.

Two types of experimental observation have a direct bearing on this question. The first, involving the measurement of rates of incorporation of various nucleic acid and protein precursors into the ribonucleoproteins of nucleus and cytoplasm, indicates that such substances are subject to a much greater metabolic flux in the former cellular compartment. As shown in Figure 93, nuclear RNA attains a much higher specific radioactivity than does cytoplasmic RNA when an animal is administered radioactive phosphate. Further, the peak of radioactivity is attained much more rapidly in the nuclear material. Thus, although the amount of RNA in the nucleolus is generally relatively small in comparison with that in the cytoplasm, the rate of turnover of the former is such that it might serve as a precursor of at least part of the cytoplasmic RNA.

A second type of approach has been made by Ficq and her col-

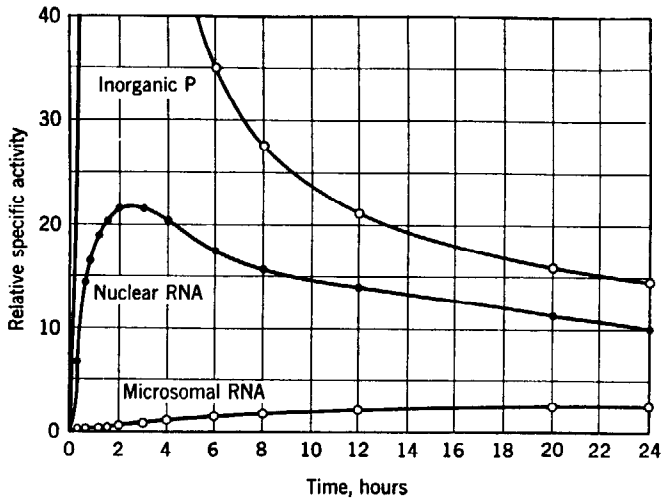

Figure 93. Relative specific activities of inorganic phosphate, nuclear RNA, and microsomal RNA of mouse liver tissue at various times after the administration of P^{32}. Redrawn from R. M. S. Smellie, *The Nucleic Acids*, volume 2 (E. Chargaff and J. N. Davidson, editors), Academic Press, 1955.

leagues,[5] using autoradiographic methods. After administration of radioactive glycine to rats and to starfish oöcytes, she observed that the rate of labeling of nuclear proteins was considerably higher than that for cytoplasmic proteins. In the starfish oöcyte the well-defined nucleolus showed particularly marked activity. Much of this radioactivity is rapidly washed out of nuclei when they are isolated from homogenized tissues.

These observations are consistent with the idea that cytoplasmic ribonucleoprotein stems from the nucleus. As usual, however, there is another side to the coin. Brachet and Szafarz have shown, for example, that cytoplasmic RNA of *Acetabularia* continues to incorporate the purine precursor, orotic acid, in spite of previous enucleation.[5] Further, Moldave and Heidelberger[6] have found that the ribonucleic acids of the nuclei, microsomes, and mitochondria show different chemical properties, such as variations in the ratios of heterocyclic bases and in nucleotide sequences. We are obviously far from an answer to the question of the essentiality of direct nuclear control of protein synthesis. Whether the present confusion is attributable to the presence of two distinct types of protein synthesis, one nuclear and one cytoplasmic, as has been suggested by Brachet and others, or whether experimental inconsistencies are due to the occurrence of

processes in enucleated and otherwise disturbed cells that represent the dying gasps of a partially functional but abnormal mechanism, is a source of some of the more exciting speculations in the field.

Some very recent work does seem to offer strong support for the latter alternative. Stich and Plaut have examined the potentials for growth, protein synthesis, and differentiation of nuclear and anuclear halves of *Acetabularia* after exposure to ribonuclease action. Their results, summarized in Figure 94, show that, in the absence of a nucleus, normal growth and synthesis cannot be resumed after RNA degradation. Stich and Plaut suggest that the nuclear product which effects the recovery of nucleated halves is RNA, but they emphasize that this suggestion should not be extended to include *all* the cytoplasmic RNA but may apply only to a small and highly critical fraction produced by the nucleus. With this reservation, these observations are in good agreement with the results of Moldave and

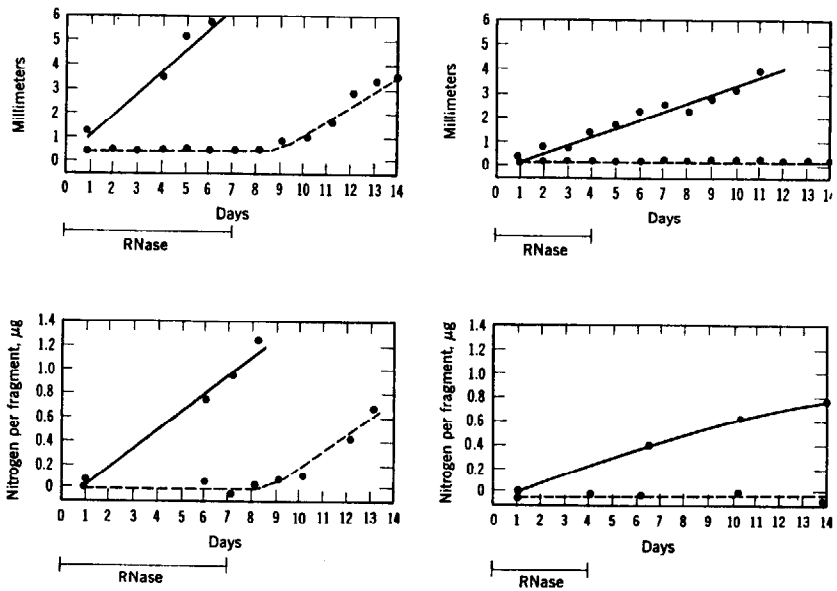

Figure 94. The effect of ribonuclease action on the growth and protein synthesis of nucleated and enucleated cell fragments of *Acetabularia mediterranea*. The top curves (left, nucleated; right, enucleated) show the effects of ribonuclease on growth and the bottom curves those on protein synthesis. Fragments were transferred to normal medium at the times indicated by the bars at the bottom of each graph. In the absence of a nucleus, normal growth and synthesis could not be resumed after RNA degradation. From H. Stich and W. Plaut, *J. Biophys. Biochem. Cytology*, 4, 119 (1958).

Heidelberger already mentioned and also with studies recently reported by McMaster-Kaye and Taylor,[7] who show that what has hitherto been referred to as "nuclear" RNA may be divided into nucleolar and chromosomal fractions, only the former of which has an especially dynamic metabolism. It would begin to seem that the nucleolus may, as Caspersson suggested, occupy a very central position in the over-all process of protein biosynthesis.

The third step in Caspersson's scheme involves the production of cytoplasmic proteins by a process dependent on the ribonucleoproteins, whose possible origin we have just discussed. Here we seem to be on reasonably firm ground, at least from the biochemical point of view. It has long been known that the rate at which a tissue can synthesize protein is correlated with its RNA content. Studies with the ultraviolet microscope and with conventional staining methods demonstrated, rather early, that such actively proliferating tissues as root tips and yeast cells and such secretory cells as the exocrine cells of the pancreas and liver were all rich in RNA. It was also observed that enucleation of amoebae caused a rapid fall in the level of RNA, paralleled by a fall in the rate of labeled amino acid incorporation.

The most direct evidence for the essential role of RNA in protein synthesis comes from studies on isolated cellular particles. When the tissues of an animal which has been administered a radioactive amino acid are homogenized and subjected to differential sedimentation, it is found that the proteins of the microsomes have become most rapidly labeled. (We must keep in mind, however, the observations of Ficq on the extractability of protein from the isolated nucleus when appraising data obtained on isolated cell particles in general.) Subdivision of microsomal matter into arbitrary fractions by differential extraction showed that those fractions containing the highest levels of RNA were also most rapidly labeled, although, in later samples, radioactivity had been rapidly transferred to the lipoprotein matrix to which the ribonucleoprotein is attached or in which it is dissolved.

The endoplasmic reticulum disappears during mitosis, as well as during starvation, as we have discussed earlier, and it is of interest in connection with Caspersson's general hypothesis that this behavior is shared by the nucleolus, which we have indicated might be implicated as a central site of ribonucleoprotein synthesis. The parallel disappearance and reappearance of ribonucleoprotein-rich reticulum on the one hand, and of the ribonucleoprotein-producing nucleolus on the other, furnish inferential evidence for the idea that these two

cellular components are somehow linked in the process of establishing information-rich biosynthetic machinery in the cytoplasm.

Protein Synthesis in Ruptured-Cell Preparations[8]

Tissue homogenates, although they incorporate amino acids only weakly and lack the integrated information system of the cell, have the advantage that the need for certain essential cell components becomes emphasized. Various cofactors or substrates may then be screened for their capacity to stimulate the synthesis of proteins.

It was observed, in a number of preliminary studies, that homogenates could support the incorporation of labeled amino acids into proteins when ATP was added as an energy source. Mitochondria, which serve as the site of ATP production through oxidative phosphorylation, could substitute for added ATP in large part. The incorporated amino acids in such systems are found mainly in the microsome fraction of the homogenate (although mitochondria would also appear to support the synthesis of some proteins, e.g. cytochrome c).

A major contribution was made by P. C. Zamecnik and E. B. Keller[9] when they observed that, if more gentle homogenizing techniques were employed, a reconstructed system consisting of microsomes and a particle-free supernatant would incorporate labeled precursors when supplied with an energy source such as phosphocreatine or phosphoenolypyruvate. This incorporation was specific for the natural L-amino acids and was abolished by the addition of ribonuclease. The true synthesis of peptide bonds was demonstrated by the isolation of peptides from partial hydrolysates of the protein which contained C^{14}.

Pursuing the dissection of this system, Zamecnik[8] and his colleagues found that a protein fraction could be prepared from the supernatant by precipitation at pH 5. This fraction no longer contained bound nucleotides, which were present in the original crude extract, and could support amino acid incorporation only when ATP and either guanosine triphosphate or guanosine diphosphate were added.

It was suggested by F. Lipmann[8] in 1941 that amino acids might be raised to an energy level, sufficient to permit them to undergo peptide bond condensations, by conjugation with high-energy phosphate groups. Support for this prediction was obtained by M. Hoagland and others,

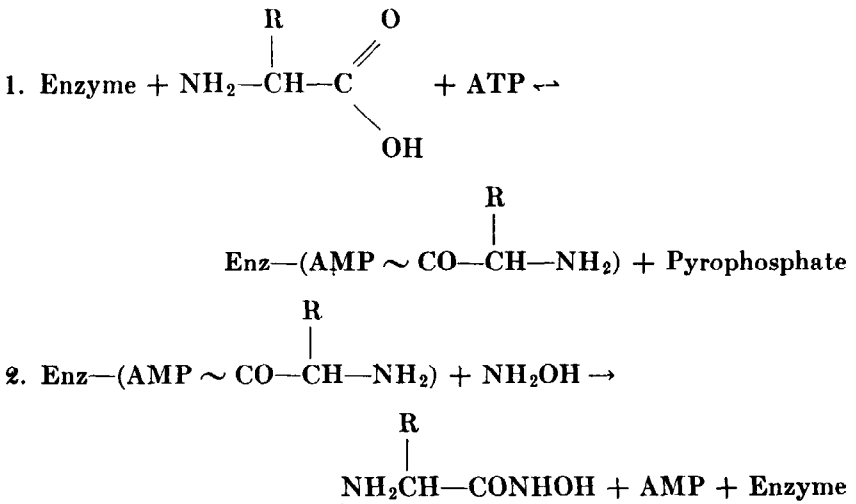

Figure 95. A postulated mechanism for the activation of amino acids for protein synthesis. In step 1 the amino acid is converted to an enzyme-bound acyl phosphate derivative of adenylic acid (AMP). This complex may be utilized for intracellular protein synthesis or, as shown in the figure, may be reacted with hydroxylamine to yield the amino acid hydroxamate.

who showed that during the incubation of amino acids with ATP and an enzyme fraction prepared from the "pH 5 enzyme" derivatives were formed which had the characteristics of an active acyl compound; that is, they formed hydroxamates upon treatment with hydroxylamine.[8] Although the active intermediate compounds have not been isolated in more than trace amounts, the fact that hydroxamates are formed and that pyrophosphate is liberated during the reaction strongly suggests that the chemical events may be described by the formulas in Figure 95. Several investigators have recently synthesized the postulated amino acid adenylates and report that they can undergo *nonspecific* condensations with pre-existing free amino groups in proteins. The decision whether AMP-amino acid conjugates are real, or only apparent, participants in the pathway between free amino acid and protein must therefore depend on future research. The postulated reactions are appealing because of their analogy to already proven activation reactions for other substrate molecules, such as fatty acids. They are also an attractive possibility for the simple reason that enzymes which form amino acid adenylates *do* exist in the cytoplasm of cells.

Perhaps the greatest puzzle in protein biosynthesis is the question of how sequences are determined and how cross-linking and folding of peptide chains is brought about in a way that yields biologically specific proteins. It seems likely that much of this process must involve RNA, since this is the only obvious substance in the cytoplasm which bears some structural (and probably metabolic) relationship to the DNA of the nucleus, from which most of or all the information that specifies the patterns of the protein molecules must originally stem. Indeed, a number of investigators have recently obtained suggestive evidence for the involvement of a soluble, nonmicrosomal, RNA fraction of the cytoplasm which is extremely active in incorporating labeled amino acids and which may act as an intermediate transport system between activated amino acids and the ribonucleoproteins of the endoplasmic reticulum.*

If we assume that RNA serves as some sort of "template" for the assembly of proteins, must there be a separate ribonucleoprotein "template" for each cellular protein, or is it possible that there exists some facet in protein synthesis which permits the utilization of common information for the synthesis of many different kinds of protein molecules? The evidence we have for the presence of repeating patterns in protein biosynthesis is, at present, very meager but suggestive enough to make the idea worthy of serious consideration. Several groups of investigators have gone to considerable trouble in assembling the known amino acid sequences in proteins (making up a total of some 400 or 500 residues) and subjecting their sequential arrangement to a rough statistical analysis for the occurrence of "repeats." As yet, no definite pattern has emerged from such efforts, although certain special sequences are found with surprising frequency. The dipeptide sequence, Ser.Arg, for example, has been demonstrated in ribonuclease, chymotrypsin, lysozyme, salmine (twice), and phosphorylase (and this statement is based on only a cursory search of the literature on protein structure). The phosphoproteins so far examined involve phosphoserine-containing sequences with remarkable similarity in structure. Table 16 contains a list of some of these, derived from proteins with no obvious connection except, perhaps, the fact that they are all secretory proteins. In every

* Several articles on the chemical nature of intermediates in protein synthesis and on the kinetics of the assembly of proteins are listed at the end of this chapter.[11, 12, 13, 14] These aspects of protein biosynthesis are under active study in many laboratories, but the field is still too confused for constructive discussion.

case the phosphoserine residue is either preceded or followed by a dicarboxyic amino acid.

Even more striking is the similarity in the sequence of amino acids associated with the "active centers" of a number of enzymes. A large number of enzymes have been found to be sensitive to reagents of the sort typified by the compound, di-isopropylfluorophosphate (DFP). Such reagents presumably inactivate by reacting with specific serine hydroxyl groups to yield the O-di-isopropyl phosphate derivative (DIP protein). After partial hydrolysis of DIP proteins, the acidic phosphopeptides may be isolated and characterized. Four enzymes, trypsin, chymotrypsin, phosphoglucomutase, and thrombin, have been studied in particular detail in relation to the DFP reaction, and it has been found that the amino acid sequence in the neighborhood of the tagged serine residue is probably identical in all four cases.[10] Although the complete sequence has been established unequivocally for only two of the proteins, it is almost certain from the preliminary data that all four involve the sequence

TABLE 16

Amino Acid Sequences Containing O-Phosphorylserine (SerP)[a]

Pepsin	Thr.SerP.Glu
Egg albumin	Glu.SerP.Ala
	Asp.SerP.Glu.Ileu.Ala
α-Casein	SerP.Glu
	SerP.Ala
	Glu.SerP

[a] A general discussion of phosphorus-containing proteins is given by G. Perlmann, *Advances in Protein Chemistry*, volume 10, (M. L. Anson, K. Bailey, and J. T. Edsall, editors), Academic Press, 1955.

Gly.Asp.SerP.Gly.Glu.Ala

in which the phosphate group is derived from the phosphorylating reagent except in the case of phosphoglucomutase, which contains phosphate as an integral part of the enzyme. The presence of an identical sequence of amino acids in these four proteins, particularly in association with areas of the molecules that are strongly implicated in catalytic function, is almost too much to attribute to chance alone.

Another example of similarity in sequence is found in the hormones of the pituitary gland whose structures were discussed in Chapters 6 and 7. The formulas for adrenocorticotropic hormone (ACTH) and for two forms of the melanocyte stimulating hormone (MSH) of porcine pituitary tissue are shown in Figure 74 (page 153). The material known as β-MSH contains a heptapeptide sequence which is identical with residues 4 to 10 of ACTH. An even more striking example of recurring sequence is α-MSH, which has a structure identical with the first thirteen residues of ACTH except for the addition of a *C*-terminal amide group and an *N*-terminal acyl radical. The similarities in these structures are particularly interesting because of the fact that ACTH is formed in the posterior lobe of the pituitary gland, whereas MSH appears to be synthesized in the *pars intermedia*.

The determination of amino acid sequences in proteins and polypeptides is still at an early stage, and unfortunately we lack sufficient data for a proper statistical analysis of the results. Nevertheless, the data we do have make it tempting to postulate that the "templates" responsible for the biosynthesis of peptide chains involve common sets of "instructions" which correspond to recurring chemical fine structure in the genetic material of the nucleus. Alternatively, various common peptide intermediates that have structures suited to specific functional requirements might be involved in the assembly of different classes of proteins. These hypotheses are attractive from the standpoint of evolutionary theory, which suggests to us a common origin for living things, both present and past and a gradual process of change in variety and kind through the modification of the genotypes available at any moment. This thesis, which is discussed in greater detail in the following chapter, would propose that "primitive" chemical structures having generalized enzymatic or hormonal functions were modified during evolution to yield families of more specialized molecules.

REFERENCES

1. F. S. Sjöstrand and V. Hanzon, *Exptl. Cell Research*, **7**, 393 (1954); *ibid.*, p. 415.
2. M. Swerdlow, A. J. Dalton, and L. S. Birks, *Anal. Chem.*, **28**, 597 (1956).
3. T. Caspersson, *Cell Growth and Cell Function*, Norton, New York, 1950.
4. K. Felix, H. Fischer, and A. Krekels, *Progress in Biophysics*, volume 6, Pergamon Press, London, p. 1, 1956.
5. For a discussion of these, and related studies, see the review by J. Brachet in *The Nucleic Acids*, volume 2 (E. Chargaff and J. N. Davidson, editors), Academic Press, New York, 1955.
6. K. Moldave and C. Heidelberger, *J. Am. Chem. Soc.*, **76**, 679 (1954).
7. R. McMaster-Kaye and H. J. Taylor, *J. Biophys. Biochem. Cytology*, **4**, 5

(1958). (The reader should be cautioned that a direct relationship between nuclear and cytoplasmic RNA is far from established—see for example, J. W. Woodward, *J. Biophys. Biochem. Cytology*, **4**, 383 (1958).
8. Reviewed in a series of papers in *Proc. Nat. Acad. Sci. U.S.*, **44**, No. 2 (1958).
9. P. C. Zamecnik and E. B. Keller, *J. Biol. Chem.*, **209**, 337 (1954).
10. J. A. Gladner and K. Laki, *J. Am. Chem. Soc.*, **80**, 1263 (1958).
11. R. Loftfield, in *Progress in Biophysics and Biochemistry*, Pergamon Press, London, 1958.
12. S. Spiegelman in *The Chemical Basis of Heredity* (W. D. McElroy and B. Glass, editors), Johns Hopkins Press, Baltimore, 1957.
13. J. L. Simkin and T. S. Work, *Nature*, **179**, 1214 (1957).
14. D. Steinberg, M. Vaughan, and C. B. Anfinsen, *Science*, **124**, 389 (1956).